Faculty of Resource Science and Technology

A LAB SCALE STUDY ON THE EFFECT OF WASTE LUBRICATING OIL TO RED TILAPIA, *Oreochromis* sp. JUVENILES

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Bachelor of Science with Honors
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Declaration

I hereby declare that the work in this project is my own except for quotations and summaries which have been fully acknowledged. No portion of the work referred to in this dissertation has been submitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

Sa'diah

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ABSTRACT

Waste lubricating oil is defined as used lubricating oil removed from internal combustion engines. Among the sources of this chemical are vehicles, mining, agricultural and construction equipment. Rapid industrialization, urbanization and transportation brought environmental risks to aquatic organism and human health due to improper disposal of waste lubricating oil into environment**.** A lab scale study was conducted with aimed to determine the effect of waste lubricating oil on red tilapia juveniles, *Orechromis* sp. in static system. Juveniles were exposed to four different concentration of waste lubricating oil (50 ml, 90 ml, 120 ml and 200 ml) for 96 hours. The lethal concentration, LC_{50} that caused 50% mortality of *Oreochromis* sp. was 91.20 ml. There was significant difference in the effects of waste lubricating oil on the mortality rate of *Oreochromis* sp. between all treatments. The result showed the swimming performances of fish were reduced after 24 hours exposure to concentration 120 and 200 ml of waste lubricating oil. Severe inflammation was observed on the kidney tissue of exposed fish after 96 h experiment. This study used only *Oreochromis* sp. juvenile; therefore future study should also use other fish species. It is also recommended to carry out this toxicity test in natural environment.

Key words: waste lubricating oil, *Oreochromis* sp., juveniles, mortality, toxicity

ABSTRAK

Sisa minyak pelincir ditakrifkan sebagai minyak pelincir yang digunakan dan dikeluarkan dari enjin pembakaran dalaman. Antara sumber-sumber bahan kimia ini adalah kenderaan, perlombongan, pertanian dan peralatan pembinaan. Perindustrian yang pesat, pembandaran dan pengangkutan membawa kepada risiko terhadap hidupan akuatik dan kesihatan manusia disebabkan oleh pelupusan tidak wajar sisa minyak pelincir ke alam sekitar. Satu kajian skala makmal telah dijalankan dengan bertujuan untuk menentukan kesan sisa minyak pelincir dalam kalangan tilapia merah muda, Oreochromis sp. dalam sistem statik. Ikan muda tersebut telah diletakkan kepada empat konsentrasi sisa minyak pelincir yang berbeza (50ml, 90ml, 120ml dan 200ml) selama 96 jam. Konsentrasi yang menyebabkan 50% kematian ikan, Oreochromis sp. adalah 91.20 ml. Terdapat perbezaan yang signifikan dalam kesan sisa minyak pelincir pada kadar kematian Oreochromis sp. Hasilnya menunjukkan aktiviti ikan ketika berenang berkurangan selepas 24 jam pendedahan kepada konsentrasi 120 dan 200 ml sisa minyak pelincir. Sisa minyak pelincir mempunyai kesan pada buah pinggang ikan ujian. Keradangan teruk telah diperhatikan pada tisu buah pinggang ikan ujian selepas 96 jam pendedahan pada sisa minyak pelincir. Kajian ini hanya menggunakan Oreochromis sp. muda, oleh itu kajian masa depan juga perlu menggunakan spesies ikan lain. Ia juga disyorkan untuk menjalankan ujian ketoksikan ini di dalam persekitaran semula jadi.

Kata Kunci: *sisa minyak pelincir, Oreochromis sp., juvana, kematian, ketoksikan*

1.0 Introduction

Waste lubricating oil, or called waste engine oil and used oil, is usually discharge from engine parts of motor vehicle such as cars and motorcycles, heavier vehicles; buses and truck, marine transportation; ships, boats and aircraft. Lubricant oil is widely used to lubricate engine parts (John and David, 2009). Lubricant also used in agricultural, industrial and mining industry to lubricate the equipment and machines. Its function is to clean, improve sealing, prevent corrosion, and cool the engine by removing all heat away, has finite lifetime and need to change to protect the engine. A high amount of waste lubricating oil is discharge from million numbers of vehicles due to maintenance activities (Faizul *et al*., 2012). This liquid is brown to black in color; found in aquatic bodies as a resulted from uncontrolled release of waste engine oil into drainage channels and canals (Ayoola *et al*., 2012).

Red nile tilapia, *Oreochromis* sp. are among the important freshwater fish in the world. In Malaysia, red tilapia contributes approximately 90% of the total tilapia production. Tilapias are hardy species and have more resistant to unfavorable water quality compared to other freshwater fish. It has ability to tolerate to low dissolved oxygen, wide range of salinities and high levels of ammonia. Since the ancient Egyptian days, it has been contributing to the world aquaculture production and remains a major freshwater species to be cultured (Amal and Zamri, 2011).

Malaysia is gradually shifting to an industrialization country and urbanization is taking place as its continue to expand rapidly in order to achieve national agenda to become a fully- developed nation by the year 2020. Both industrialization and urbanization have environmental risks. Department of Environment Quality stated that waste oil is classified as scheduled wastes under the First Schedule of the Environmental Quality

(Schedule Wastes). However, since the enforcement of this law is lacking, solving waste lubricating oil problem is a challenge. Awareness of waste lubricating oil pollution should be raised among the public and private sectors especially from automotive industries. Some studies about the waste lubricating oil spill had been carried out in Malaysia, for example design of Intelligant SoC Controller for engine oil sensing and monitoring system by Faizul *et al*. (2012). However, there is no publication on the effect of waste lubricating oil on *Oreochromis* sp. juveniles in Malaysia. Therefore, this study is designed to:

i. determine the effect of waste lubricating oil toxicity on red tilapia juveniles, *Oreochromis* sp.
ii. assess the behavioral response (swimming performance) of juveniles *Oreochromis* sp. during exposure to waste lubricating oil.

Upon completion of this study, data obtained could be used as baseline data to raise awareness among stakeholders of lubricating oil and other related research in future.

1.0 Literature Review

This section is divided into 4 main topics namely waste oil spill, effect of waste oil spill, toxicity test and behavioral responses of test organisms.

2.1 Waste oil spill

About 150 million liters of waste lubricating oil is produce annually in Malaysia (Faizul *et al*., 2012). Global transportation of goods is dominated by ships. Ships usually travel with oil, fuels and engine lubricants which sometimes is deliberately discharge into the sea. Oil spill into the ocean from ships could also be an accident (Khan and Singh, 2011). In Malaysia, Straits of Malacca is susceptible to marine pollution due to its major commercial shipping route between Indian Ocean and Pacific Ocean. High traffic volume causes uncontrolled vessels discharges such as deballasting, tank cleaning, bilging and bunkering. These activities contribute to oil and grease spill into marine water. Furthermore, the risk of vessels accidents and marine pollution is expected to be increase as traffic volume is higher with expansion of world trade (Maizatun and Mariani, 2011).

Nigeria is one of the countries that faces serious problem in water resources related to pollution of waste oil spills (Ayoola *et al*., 2012; Ogbuehi *et al*., 2011; Thongra-ar *et al*., 2003). Waste lubricating oil is found after process of servicing used oil from automobile and generator engines. In Nigeria, it is common to discharge of waste oil into gutters, water drains, open vacant plots and farms which is mainly done by auto mechanics and allied artisans with workshop on the roadside and open places (Agbogidi, 2010).Irresponsible people dispose the waste engine oil to anywhere which is convenient spot for them to dump the waste oil indiscriminately such as in wooden areas, in road side ditches, storm drain and even into near creeks or rivers as they are lack of awareness and concerns on the environmental destruction (Ogbuehi *et al*., 2011).

2.2 Effect of waste oil spill

According to official website of Department of Environment Ministry of Natural Resources and Environment Malaysia, waste oil contain hazardous contaminants such as PAHs, PCBs, arsenic, cadmium, copper, magnesium and others, which can induce illness and diseases in human and living organism through inhalation, ingestion and skin contact. These contaminants are environmentally hazardous. They can pollute groundwater and therefore water supply in that area is not safe for drinking (Nasir *et al*., 2011).

Oil spill is a serious threat to plants and animals. Marine species can be affected by oil pollution with concentration as low as one ppm. When oil is spilled into the seas, aquatic organisms would ingest hydrocarbons through the food chain and bioaccumulation occurs in their tissue for a period of time. Human will be affected by this toxicant when they consume food polluted by hydrocarbon (Norazida *et al*., 2011). Waste pollutants carried by run-off during rainfall to nearby farms will become widespread. The agricultural areas located near to workshops are the worst hit during rainfalls (Ikhajiagbe and Anoliefo, 2011). Furthermore, waste engine oil has caused adverse impacts on aquaculture fisheries in Malaysia (Norazida *et al*., 2011).

Waste engine oil not only affects fish in terms of mortality, but also affects the soil properties, plant growth, germination of seeds and survival of microorganism. Agbogidi (2010) had studied about the response of six cultivars of cowpea, *Vigna unguiculata* to waste engine oil. The seeds soaked in the oil for 16 hours failed to germinate. No germination was recorded in cowpea seeds soaked in oil for more than 2 hours. Njoku *et al*. (2012) had studied the effects of application of waste engine oil on the growth and performance maize plant, *Zea mays* showed that the growth of maize plants is decreased with the increased concentration of waste engine oil. Sharifi *et al*. (2007) had carried out study on the germination and growth of six plant species on contaminated soil with waste oil showed that all species to the contaminated soil. The result showed the reduction in germination, above ground height and biomass for all species were significantly ($P<0.05$) different when compared to the controls.

Limited information and studies regarding the effect of chronic exposure of hydrocarbons such as waste lubricating oil on terrestrial animals was the objective for the study of the hepatotoxic effect of waste lubricating oil in Wistar albino rats by Patrick (2010). Based on his study, the exposure of rats to different concentration of waste lubricating oil had resulted to a significant hepatic damage and the liver damage in the rats after the toxicity experiment.

2.3 Toxicity Test

Toxicity test is used to examine the toxic effects when a chemical is absorbed in organism, via mouth, skins and lungs (Deora *et al*., 2010). It is used to determine the susceptibility and survival potential of organisms to particular toxic substances (Neukobin *et al*., 2012).

Acute toxicity is used to determine the effect of substance induced in an organism within a short time (days) of exposure to a substance. There are several terms used regarding to toxicity test definiton namely LC_{50} and LD_{50}. LC stands for 'Lethal Concentration' and LD stands for 'Lethal Dose'. This test is used to determine the concentration of a chemical that will kill half of the sample populations of test organism in a specified time through exposure to toxicant (Agbogidi, 2010).

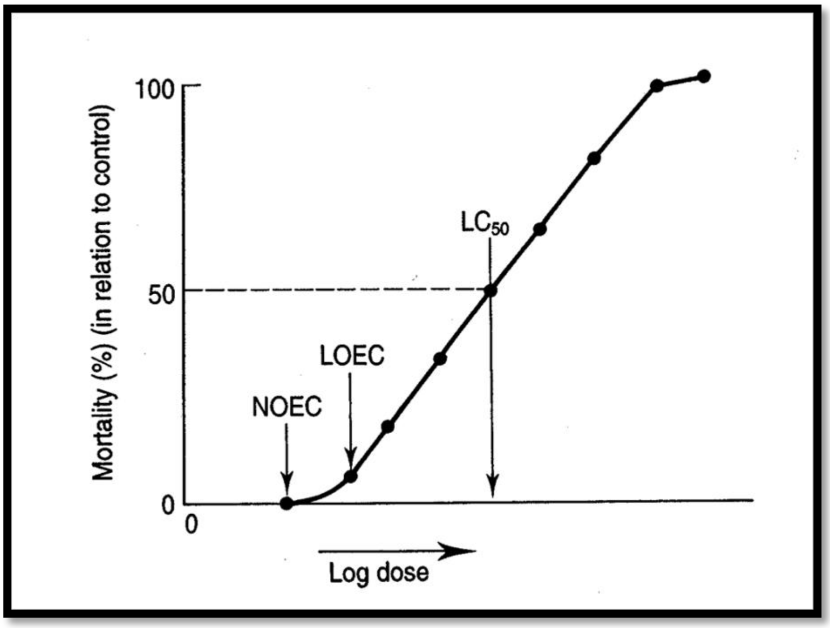

Figure 2.1: Graph of relationship between dose and response of an organism toward toxicant (adapted from *Principles of Ecotoxicology*, 2006).

In toxicity test, it is also possible to determine the highest concentration or doses that cause no toxicity effect, the no observed effect concentration (NOEC) and low observed effect concentration (LOEC) respectively (Walker *et al*., 2006). These points is illustrated in Figure 2.1 which refers to the determination of LC_{50} after 96 h exposure towards toxicant.

2.4 Test Organism

Fish is used because its biological character; big body size compared to algae, crustacean and mollusk, long life cycle, and easy to culture in the laboratory. Fish is placed at the top position in aquatic food chain and human health will be directly affected (Zhou *et al*., 2008). Therefore, much attention had been given to juvenile fish because of their small size and more sensitive to toxicants compared to adult fish (Ugwu *et al*., 2011).

2.5 Behavioral responses

The test organism in experiment is monitored within 96 h of oil exposure for mortality and swimming performances (Ugwu *et al*., 2011). Fish which lost their equilibrium, floated ventral side up and did not respond to touch, were considered dead during toxicity test (Vincent *et al*., 2010).

2.6 Changes in fish kidney

According to Ayoola and Alajabo (2012), kidney is used as the key important organ which showing the effect of chemical on the excretory system. Once the chemical is absorbed into organism, the substance in the chemical is transported to various tissues in organism by blood. They had carried out the study on the hispathological effects of lubricating oil on Black Jaw tilapia, *Sarotherodon melanotheron* and the result showed that inflammation had occur in test fish kidney.

3.0 Materials and Method

3.1 Test Organism

Juveniles of red tilapia, *Oreochromis* sp. with average length of 5.5 ± 0.2 cm were used in this experiment. All test fish were obtained from Fisheries Research Institute Gelang Patah, Johor for toxicity test. While Koi juveniles, *Cyprinus carpio* with average length 2.1 ± 0.1 cm kindly donated by Prof Dr. Lee Nyanti were used during preliminary test.

3.2 Source of waste toxic

The waste lubricating oil used for preliminary test was obtained from workshops at Kota Samarahan area. For toxicity test experiment oil was obtained from workshops in Johor Bahru area.

3.3 Acclimatization of fish juveniles

All test organisms were acclimatized under laboratory conditions. A total of 200 juveniles of *Oreochromis* sp. were acclimatized at Drilling Laboratory, Faculty of Petroleum, University Technology Malaysia for 6 days prior to the toxicity test experiment.

All test fish were placed in big container comprising 100L of de-chlorinated tap water following Thongra-ar *et al*. (2003). The acclimatization stage was conducted at ambient temperature (28-30°C) with photoperiod of 12h light 12h dark and dissolved oxygen concentrations were maintained between 60-100% saturation with the aeration system. Juveniles were fed *ad libitum* with commercial pellet twice a day during acclimatization stage.

Table 3.1: Description of treatments that were used for toxicity test in this study.

Tank (No.)	Treatment	Concentration of Waste Lubricating Oil (ml)
1-3	A	50
4-6	B	90
7-9	C	120
10-12	D	200
13-15	Control	No oil added

3.4 Experimental Design

For toxicity experiment, 120 juveniles of *Oreochromis* sp. were exposed to four different concentrations (50ml/L, 90ml/L, 120ml/L and 200 ml/L) of waste lubricating oil as in Table 3.1. For each experiment, one control was included. Each container was filled with 2 L of tap water. After that, various concentration of waste lubricating oil was added. Later, eight juveniles of *Oreochromis* sp. were placed into each container. An illustration of this experiment is shown in Appendix A. The experiment had included 3 phases namely documenting the mortality of test fish, observing the fish responded changes and changes in test fish kidney after 96 h exposure to waste lubricating oil.

3.5 Toxicity exposure

All test fish in all replicate containers were left for 96 h in different concentrations of oil. All the test fish were monitored within 96 h of oil exposure for mortality and behavioral changes. Fish mortality was observed every 24 h and the fish juveniles was consider being death as they remain immobile and sink on the bottom of the container (Rodrigues *et al*., 2000).

3.6 Ambient water quality parameter monitoring

Selected ambient water quality parameter namely water temperature, pH and DO using Hanna HI9810, Eutech and Hanna HI9142 respectively. Data were was recorded at every 24 h for all replicates of each treatment.

3.7 Changes in test fish kidney

The kidney of the dead test fish was dissected and preserved in 10% buffered formalin after dissects from test fishes that had died after the experiment (Ayoola and Alajabo, 2012). The changes in test fish kidney between the control and the treatment was observed under research microscope, model Nikon Eclipse 80i.

3.8 Data Analysis

LC_{50} of toxicity test was analyzed by using Probit analysis. It transforms the mortality rate (%) of test fish with Probit value. The result obtained from Probit analysis revealed the concentration of waste lubricating oil required to kill a certain proportion of test fish. Selected ambient water quality (DO, pH and temperature) were recorded. Data was further analyzed using one-way ANOVA and Tukey Test (if necessary) to determine whether there are any differences between treatment. Data was analyzed by using software SPSS version 16. Flow chart summarizing work involved in this study as in Appendix B.

4.0 Result

This section comprised four subsections namely mortality of test fish, LC_{50} values, selected ambient water parameters monitoring and effect on kidney.

4.1 Mortality of test fish

The number of *Oreochromis* sp. juveniles mortality in each treatment is summarized in Table 4.1.

Table 4.1: The mortality of *Oreochromis* sp. after 96 hours during toxicity test experiment.

Each experiment began with 8 individuals of *Oreochromis* sp. juveniles.

Treatment	Concentration of waste engine oil (mL)	R1	R2	R3	Total Mortality	% Mortality
A	50	0	1	1	2	8
B	90	4	4	3	11	46
C	120	6	6	7	19	79
D	200	8	8	8	24	100
Control	0	0	0	0	0	0

Based on Table 4.1, there was no mortality in the control experiment. This is because in control, no waste lubricating oil was used. After 96 hours, Treatment D showed the highest percentage of fish mortality (100%) followed by Treatment C (79%), and Treatment B (46%). Treatment A showed the lowest mortality rate (8%).

Based on the Figure 4.1, percentage of *Oreochromis* sp. mortality was higher as concentration waste lubricating oil increased.

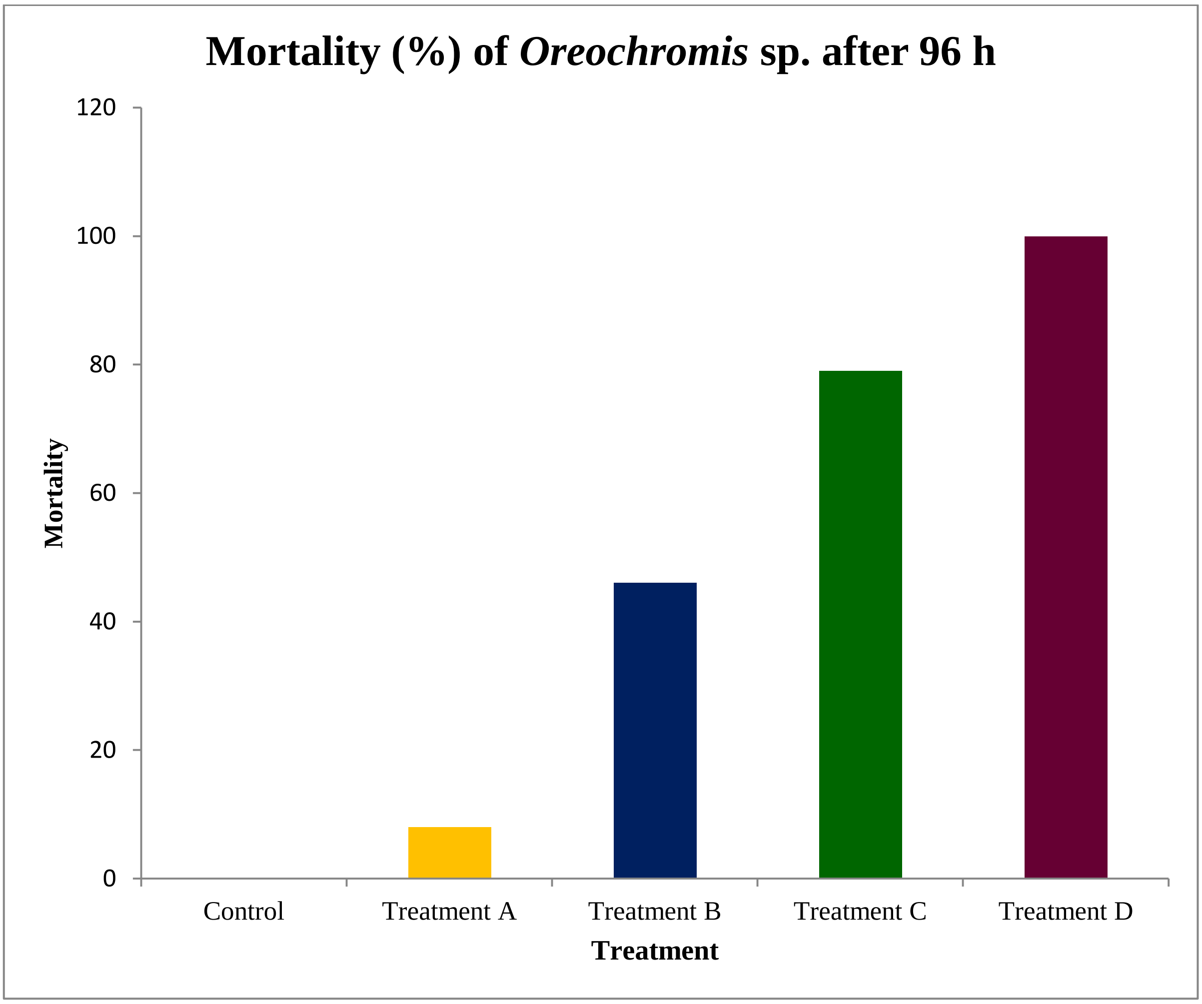

Figure 4.1: Percentage of *Oreochromis* sp. juveniles mortality during this study.

One-way ANOVA is a method used to determine whether there is any significant difference in mortality means between all treatments. Two test hypotheses were made for making decision and conclusion:

Null hypothesis: H_0: There is no significant difference in mortality means between all treatments

Alternative hypothesis: H_1: There is a significant different in mortality means between all treatments

where α=0.05

One-way ANOVA showed that there is significant difference in the means for mortality between all treatments (as shown in Table 8.1, Appendix C). Since p-value ($P = 0.000$) is less than α (0.05), the null hypothesis is rejected. It concludes that the means in mortality for all treatments are not same.

To determine whether waste lubricating oil has any influence on *Oreochromis* sp. juveniles mortality, two statistical analysis had been carried out namely Pearson Correlation and Linear regression.

Table 4.2 showed that mortality of *Oreochromis* sp. is significantly correlated with concentration of waste lubricating oil in the experiment as ($P = 0.000$) and ($r = 0.957$).

Table 4.2: Pearson Correlation analysis for concentration-mortality in this study.

		Concentration	Mortality
Concentration	Pearson Correlation	1	.957**
	Sig.		.000
Mortality	Pearson Correlation	.957**	1
	Sig.	.000	

** Correlation is significant at the 0.05 level (2-tailed).

Regression analysis showed strong positive correlation between concentrations of waste engine oil with percentage of mortality (Figure 4.2), therefore the mortality of test fish *Oreochromis* sp. juveniles during this experiment is strongly affected by the concentration of waste engine oil. The percentage of mortality is increased with the increased in concentration of waste engine oil.

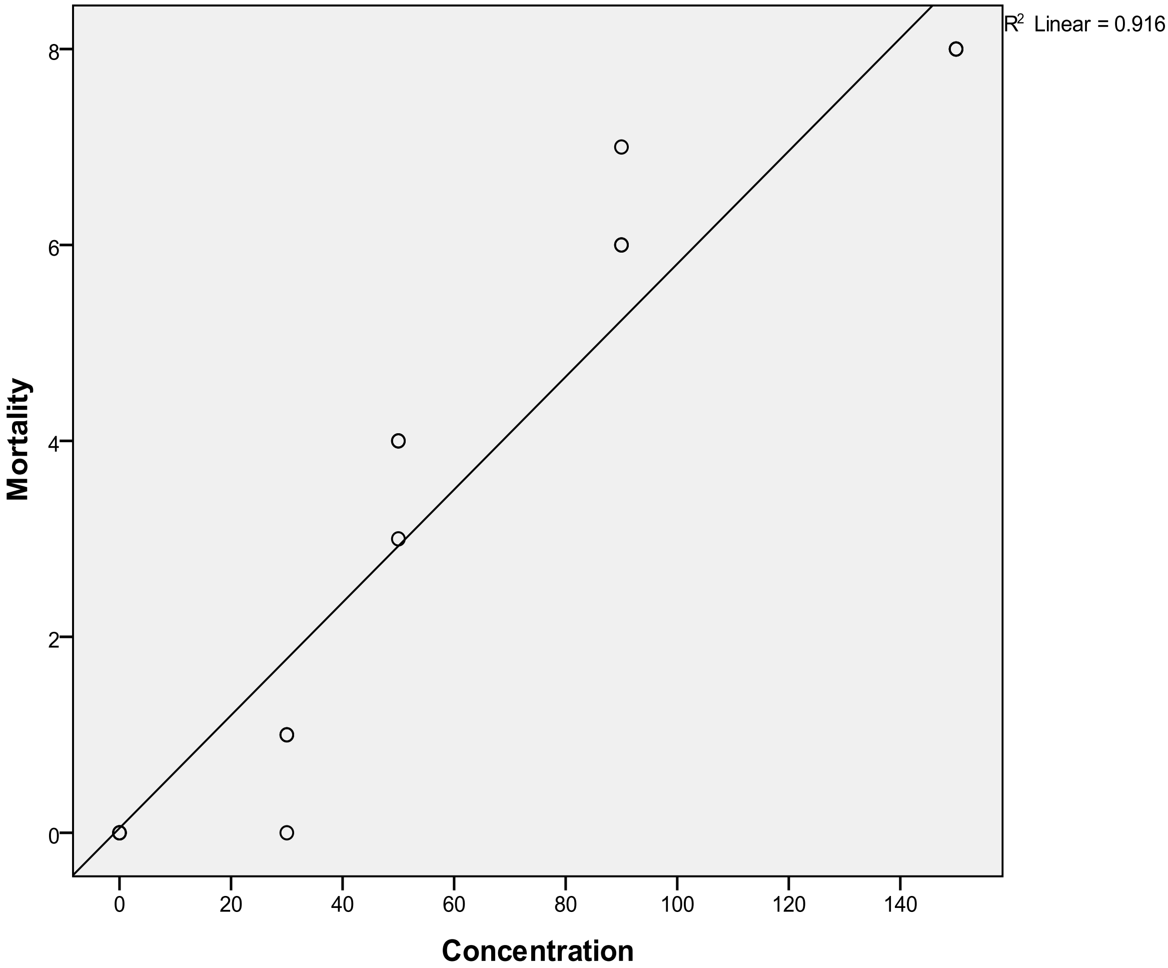

Figure 4.2: Linear regression graph for concentration waste lubricating oil-mortality of *Oreochromis* sp.